4.6 Provisional Guidance for the Initial Assessment of Health Effects*

4.6.1 Introduction

1. This document provides guidance for the initial assessment of health effects of chemicals with a full SIDS, including suggestions for making decisions on any follow-up testing if this is considered to be necessary. This document was first drafted based on relevant sections of the monographs of the International Programme on Chemical Safety (IPCS). These monographs are listed in Annex 2 to this document and can be consulted for information about making fuller assessments of chemical substances.

2. Although the SIDS is the minimum requirement for making an initial assessment of High Production Volume (HPV) chemicals in the OECD Existing Chemicals Programme, for many chemicals currently under consideration there will be data already available in excess of SIDS; these should, of course, be assessed and taken into consideration when developing conclusions and recommendations. However, in making the initial assessment of health effects, the elements in the SIDS which are relevant in this respect are:

- Acute Toxicity;
- Repeated Dose Toxicity;
- Genetic Toxicity; and
- Reproduction/Developmental Toxicity.

3. In the traditional assessment of repeated dose toxicity and reproduction/developmental toxicity, Uncertainty Factors (UF's) are used and the Estimated Level of Low Concern (EDLC) is calculated from the No-Observed-Adverse-Effect level (NOAEL) or, when not available, the Lowest Observed-Adverse-Effect level (LOAEL) derived from animal test results; however, since UF's are always chosen arbitrarily, it is often difficult for readers of assessment reports to understand the relevance of the factors used. Although this approach has been used for many years, there is no strong scientific evidence supporting its use. Therefore, at the 4th SIDS Initial Assessment Meeting in May 1996, participants agreed that UF's should not be included in assessment reports which are to be discussed and published in the OECD context.

4. Instead of using UF's and EDLC, N(L)OAEL or, if available, actual human data can be compared directly to the Estimated Human Exposure (EHE) level. This approach, the so-called "margin of safety" approach, is adopted in the EU Technical Guidance Documents for the implementation of the EC Regulation 793/93 and is described in this Provisional Guidance. Tentative general guidance for using UF's is attached as Annex 1 for possible use by Member countries in risk assessment at a national level. Both approaches have a similar end result. Whichever is used, it is important to consider both the effects and (potential) exposure of each chemical in a case-by-case manner and to apply professional judgement by, for example, toxicologists and occupational hygienists when evaluating the adequacy of any tests and the interpretation of results.

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* This document was first prepared by the OECD Secretariat based on the monographs of the IPCS. It has been updated to reflect comments by Member countries and agreements reached in the context of the OECD Existing Chemicals Programme up to May 1996.

4.6.2 Acute Toxicity

5. In the assessment of the toxicity of a chemical, the determination of the acute toxicity is often the first step. It provides information on the health effects likely to arise from short-term exposure at a relatively high dose. Substances are classified either on the basis of the approximate LD_{50} (or LC_{50}) value or a discriminating dose. Other information obtained from the acute toxicity test, such as types of effects observed, their time of onset, duration and severity, and shape of the dose-response curve (steep or shallow) are also useful in the hazard assessment (and in the design of further studies).

6. When only acute oral data are available, the need to obtain data from tests by other routes should be considered in the light of the physical-chemical properties of the chemical, the nature and magnitude of the results from the oral route study, the actual or potential routes of exposure, and the measured or estimated exposure level.

7. In the initial assessment of SIDS chemicals, data on acute toxicity will usually not lead to recommending action for follow-up testing, although exceptional findings (high lethality, neurotoxicity seen at low doses, etc.) may warrant such action.

8. When data on acute toxicity indicate that a chemical is (very) toxic or harmful, this could lead to risk management activities, such as labelling or reduction of occupational exposure. In many cases, however, recommendations for such activities would not be needed for SIDS chemicals because where acute toxicity data were known, appropriate action would have already been taken.

4.6.3 Repeated Dose Toxicity

9. One of the main objectives of any repeated dose study with a duration of administration of normally at least 28-days is to obtain a value for the No-Observed-Adverse-Effect level (NOAEL), or the Lowest Observed-Adverse-Effect level (LOAEL), based on which the estimated effect level of concern for humans will be considered. The NOAEL could also be used in identifying chemicals which could be candidates for further testing. The NOAEL is considered to be the highest daily dose or concentration of a substance at which there is no adverse alteration observed in the morphology, functional capacity, growth, development, etc. of the target. The LOAEL, on the other hand, is considered to be the lowest daily dose or concentration of a substance at which any of these adverse alterations is actually observed. In general, greater confidence for establishing an estimated effect level of concern is placed in a NOAEL than in a LOAEL; in a NOAEL obtained from a sub-chronic study rather than one from a sub-acute study; in a test which demonstrates a clear dose-response relationship; and in a test in which the manifestations of toxicity are well-defined. In principle, a NOAEL should be obtained in each repeated dose study and can be used as a marker for human effects. However, when a reliable dose-response relationship is obtained, and a NOAEL cannot be estimated, a LOAEL could be used for this purpose.

10. As an alternative to this "classical" NOAEL approach, where feasible the so-called "bench-mark dose" approach could also be adopted. However, as this latter system uses the lower confidence limit of the dose corresponding to the lowest increase judged to be toxicologically significant in the incidence of an effect, and calculated on the basis of at least two dose levels showing an effect, it is anticipated that the number of repeated dose studies where adequate quantal or continuous information is available will be limited.

11. For the initial assessment of repeated dose toxicity, it might be useful to consider each route of potential human exposure (oral, inhalation, dermal) separately assuming equal absorption rates when no specific data on actual absorption are available. If it is expected that exposure via several routes will occur, the total dose from all these routes should preferably be considered.

12. What is considered to be an adverse effect is dependent on expert judgement. In those cases where an adverse effect is observed in, for example, a parameter which monitors an organ system, such as a clinical biochemical change in a measurement of liver function, more weight can be attributed to its significance if other observations for that organ system, such as necropsy findings and to a lesser extent organ weight difference, also indicate an adverse effect. In addition, the dose response of an adverse effect, i.e. the progression of a change in an organ system with the dose, is a factor which adds weight to the significance of the effect. Further aspects which are usually considered include: reversibility of the toxicity, severity of the effect, latency of the onset of the effect and the shape of the dose-response curve.

13. For the screening of chemicals, the initial assessment can be conducted using an Estimated Human Exposure (EHE) level and the N(L)OAEL. The EHE can be obtained by using the approaches described in the Provisional Guidance for the Initial Assessment of Environmental Exposure and of Occupational and Consumer Exposure (see Section 4.3 and 4.4). If the EHE is larger than or equal to the N(L)OAEL, a hazard may exist and further testing or other activities should be considered. If the EHE is smaller than the N(L)OAEL, the magnitude of the N(L)OAEL/EHE ratio, called "margin of safety", needs to be considered taking account of the following parameters:

- Difference in exposure (route, duration, frequency and pattern)
- The nature and severity of the effect
- The dose (concentration)-response (effect) relationship observed
- The inter- and intraspecies variability
- The overall confidence in the database

Expert judgement is required to weigh these individual parameters on a case-by-case basis.

14. As mentioned above, Uncertainty Factors (UF's) have traditionally been used for initial assessment of repeated-dose toxicity (see Annex 1). To a large extent, both approaches can be considered equivalent in that they reflect the confidence of the assessor in the quality and relevance of the database for a particular endpoint. The approach used should be transparent and a justification should be provided by the assessor for the conclusion reached.

4.6.4 Genetic Toxicity

15. Testing for genetic toxicity is conducted so that chemicals may be assessed for their potential to cause transmissible damage to the genetic material of somatic cells (with potential carcinogenic consequences) and germ cells (which may result in heritable damage to the offspring). Unlike most other toxicological effects, genotoxic effects *in vivo* are generally assumed to have no exposure threshold. Thus an important factor to be taken into account when thinking about further testing and/or risk reduction is whether or not human exposure occurs or may occur and, if so, which and how many humans are (potentially) exposed and the circumstances of any such exposures rather than the levels.

16. It is also essential to differentiate between the *in vitro* tests which are primarily used to investigate intrinsic potential of chemicals to cause genetic damage and the *in vivo* tests which are often used to investigate if these intrinsic properties are expressed in whole animals, to confirm negative *in vitro* results and to study effects of metabolites. Where an *in vivo* study is not available it should be considered whether the activation system used in *in vitro* is adequate.

17. For the initial assessment, results of at least two tests for genetic toxicity will generally be provided in the SIDS. These are expected to include results of a point mutation test [e.g. an *in vitro Salmonella typhimurium*, Reverse Mutation Assay, OECD Test Guideline (TG 471)] and a chromosomal aberration test [e.g. an *in vitro* Mammalian Cytogenetic Test (TG 473) or an *in vivo* Micronucleus Test (TG 474)].

18. Various different approaches are available to assist with the initial assessment of genetic toxicity (see Tables 1, 2 and 3 for guidance proposed by the OECD, Japan and the EU respectively). The OECD approach (Table 1) is the only one to consider the scenario where one of the two initial assessment tests is an *in vivo* study for clastogenicity. A somewhat more simplified approach, recommended by Japan, is summarised in Table 2. In some cases, the results of more than the two initial tests, even standardised *in vitro* or *in vivo* germ cell tests, may be available. The general approach, as recommended by the European Commission to be used for initial assessment based on the various patterns of results, is summarised in Table 3.

4.6.5 Reproduction/Developmental Toxicity

19. Reproduction toxicity represents any effect on fertility and reproduction that can adversely affect the continuation of the species. Developmental toxicity is any adverse effect induced during the developmental period, i.e. from conception through puberty. The major manifestations of developmental toxicity include death of the developing organism, structural abnormalities, altered growth and functional deficiencies. Developmental toxicity can be considered a component of reproductive toxicity, and often it is difficult to distinguish between effects mediated through the parents versus direct interaction with developmental processes.

20. Because of the nature of the observations and variety of classes of reproduction/developmental toxicity, the following paragraphs describe in slightly more detail suggestions for the initial assessment of these studies. The organisation of the information for an assessment of reproduction and developmental toxicity is described in a number of OECD Test Guidelines related to these endpoints (TG 414, 415, 416) and the guidelines for the Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (TG 422) and the Reproduction/Developmental Toxicity Screening Test (TG 421). For example, toxic response data should be considered by sex and dose and, when possible, be sub-divided into reproductive and developmental effects. Reproduction effects would include, *inter alia*, altered fertility indices for males and females, effects on mating performance or other factors affecting reproductive function and, when related to the nursing capacity of the females, postnatal viability indices for the offspring or other postnatal signs of toxicity. Developmental effects, either as a consequence of maternal toxicity or as a direct effect on the developing organism, would include, *inter alia*, decreased numbers and percentages of live offspring per litter, and increased numbers and percentage of affected offspring (male, female or combined) per litter. Data on maternal toxicity and on certain metabolic or kinetic observations need to be considered when determining the nature, severity and relevance of developmental toxicity.

21. Reproductive and developmental effects exhibit dose-response relationships, and where these effects are not genotoxic (e.g. heritable) thresholds are generally assumed to exist. It is thus possible to estimate exposure levels unlikely to produce effects in humans on the basis of a NOAEL obtained in an animal experiment, in a similar manner to that for repeated dose toxicity.

22. The occurrence of a dose level producing well defined toxicity is considered of crucial importance in reproductive toxicity studies. This is called for in the OECD Test Guidelines for both screening tests, 421 and 422. Tests in which toxicity is not observed should, therefore, not be considered as adequate tests unless the limit concentration of 1000 mg/kg has been included.

23. In addition, useful information can be derived from the repeated dose toxicity study, e.g. pathology in the reproductive organs, if specific histological examination has been carried out and a comparison of dose-response curves for such an effect between males and females could be made both in the repeated dose toxicity and the reproduction toxicity study.

24. For the reproduction toxicity endpoint:

- when a 90-day repeated dose study is available and is sufficiently documented with respect to studying effects in the reproductive organs and a developmental study is available, the requirements for the reproduction toxicity endpoint would be satisfied;

- when either a 90-day or 28-day repeated dose study is the only repeated dose study available, it is recommended that the reproduction/developmental toxicity screening test (e.g. TG 421) be carried out in order to satisfy the requirements for the reproduction toxicity endpoint; and

- when a 90-day repeated dose study is available and demonstrates no effects on the reproductive organs, in particular the testes, then a developmental study (e.g. TG 414) can be considered as an adequate test to complete information on reproduction/developmental effect.

25. In the similar way to repeated dose toxicity, the "margin of safety" approach could be used for the initial assessment instead of using Uncertainty Factors (UF's). Guidance for the use of UF's is described in Annex 1 to this document.

26. When considering the "margin of safety" for reproduction/developmental toxicity, its magnitude can be compared to that of repeated dose toxicity. A relatively high "margin of safety" may be acceptable when severe or

irreversible developmental effects occur at exposure levels below those that induce well-defined parental toxicity; in other words when the test substance produces severe developmental effects that are not secondary to general toxicity. The same level of "margin of safety" may be acceptable when developmental toxicity is only observed at exposure levels that are also toxic to the parent animals. The same considerations for the repeated dose study should be addressed when considering "margin of safety" for reproductive/developmental toxicity. An assessment of whether the effects seen in reproduction toxicity studies are secondary to general toxicity, or are specific reproductive effects, is important and expert judgement is necessary.

27. This concept of considering higher "margin of safety" for developmental effects when they occur in the absence of maternal toxicity than when observed at levels that are also toxic to the parent animals is generally accepted. However, individual Member countries may consider developmental toxicity in the presence of maternal toxicity of as great a concern as developmental toxicity seen in its absence.

4.6.6 Suggestions for Follow-up Testing

Repeated Dose Toxicity

28. If the estimated exposure level (EHE) for a specific human population is larger than or close to the estimated effect level of concern calculated from a 28-day repeated dose study, a 90-day subchronic toxicity test (TG 408, 411 or 413) could be conducted, depending on reliability of the exposure estimation. Information on exposure should be considered in detail before deciding if this is needed. In the subchronic toxicity test, a more detailed examination of effects observed in a 28-day study would be important. If the effects found in a 28-day repeated dose toxicity test are reason for concern, and substantial long-term exposure is expected to occur, it might be more effective to consider undertaking a longer toxicity test with an exposure duration of at least six months. If there is sufficient justification for a study for "conventional" (i.e. non-carcinogenic) toxic effects of longer duration than 90 days, then it should be combined with a carcinogenicity bioassay.

Reproduction/Developmental Toxicity

29. If EHE is larger than or close to the estimated effect level of concern derived from reproduction/developmental toxicity studies at a screening level, a One- or Two-Generation Reproduction Toxicity test (TG 415 or 416), which could include peri- and postnatal administration and/or a Developmental Toxicity test (TG 414), could be considered depending on the reliability of the dose-response curve and the type of toxic effects seen. Follow-up studies which are tailored to address a specific concern might also be appropriate. For example, foetal or birth weight reduction is often the only indicator of possible developmental toxicity and could be followed up in cases of concern by studies to evaluate postnatal viability, growth and survival through weaning. Studies in a second species might also be useful.

30. When deciding to conduct a follow-up reproduction/developmental toxicity study, information on exposure has to be taken into consideration. For example, for substances which have only been tested in a reproductive screening test (e.g. TG 421 or 422) and substantial, widespread and/or prolonged exposure is anticipated, serious consideration should be given to conducting definitive reproduction toxicity tests (i.e. TG 415/416 and 414). Also SARs may be taken into account when considering further testing.

31. For both repeated dose and reproduction/developmental toxicity, when more than one route is of concern, the route giving the greatest reason for concern could be chosen for a follow-up test. Similarly, if the concern is for more than one observed effect, then any follow-up test could be tailored to study that effect having the greatest concern.

Table 1

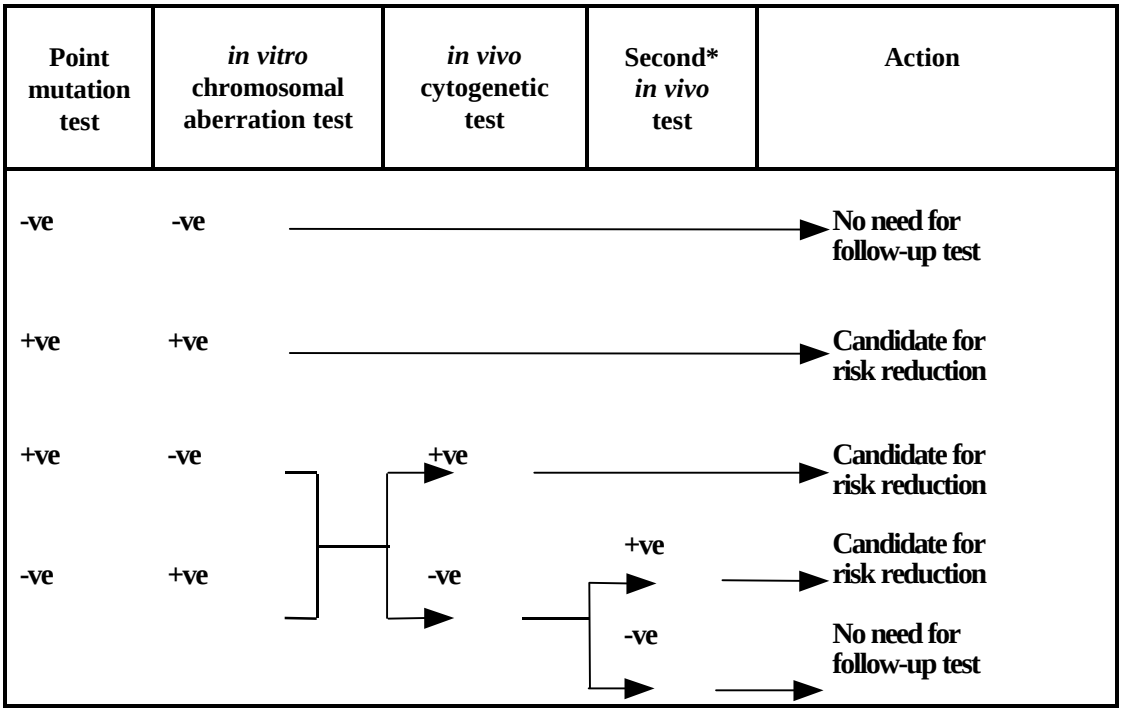
OECD Proposals for the Initial Assessment for Genetic Toxicity

	Point mutation test	*in vitro* Chromosomal aberration test	*in vivo* Chromosomal aberration test	Second *in vivo* test	Action
A	**Negative**	**Negative**			**No need for further testing**
B	**Negative**	**Positive**	**Positive**		**Candidate for in-depth review**
				Positive	**Candidate for in-depth review**
			Negative	**Negative**	**No need for further testing**
C	**Positive**	**Positive**			**Candidate for in-depth review**
D	**Positive**	**Negative**	**Positive**		**Candidate for in-depth review**
				Positive	**Candidate for in-depth review**
			Negative	**Negative**	**No need for further testing**
E	**Negative**		**Negative**		**No need for further testing**
F	**Negative**		**Positive**		**Candidate for in-depth review**
G	**Positive**		**Positive**		**Candidate for in-depth review**
H	**Positive**		**Negative**	**Positive**	**Candidate for in-depth review**
				Negative	**No need for further testing**

Note: This table assumes that the results from a point mutation (e.g. Ames type) test, and an *in vitro* (A to D) or an *in vivo* (E to H) chromosomal aberration test, are available. The arrows indicate the proposed sequence of actions following these results.

Table 2

Proposal by Japan for Initial Assessment of Genetic Toxicity



* *In vivo* liver UDS test, mouse spot test, or gene mutation test with transgenic animals.

Note: Negative in both point mutation test and *in vitro* chromosomal aberration test does not need for follow-up test. While, chemicals that give positive results in both *in vitro* tests are candidates for risk reduction. Essentially any *in vivo* positive indicates the need for risk reduction activity. In order to negate positive *in vitro* data *in vivo* negative information from at least two different tissues will be needed.

Any positive *in vivo* (also both *in vitro* positive) data might be negated by the negative data of *in vivo* germ cell test (e.g. dominant lethal test or germ cell cytogenetic test) and also the negative data of long-term carcinogenicity test.

Table 3

Proposal by the European Commission for Initial Assessment of Genetic Toxicity

- *in vivo* germ cell positive:

  No further testing. Control measures necessary. Further information on exposure may be required to assess possibility of risk by reducing exposure.

- *in vivo* somatic cell positive:

  Control measures as above. Consider testing for germ cell effects if not already done to an adequate standard.

- *in vitro* positive:

  One somatic cell test *in vivo* conducted and negative:

  Consider control measures. A second test should be conducted *in vivo* using a different somatic target tissue from the first.

- i*n vitro* positive but no testing yet *in vivo*:

  Consider control measures. Test *in vivo* in somatic cells, usually bone marrow.

- *in vitro* negative (two adequate tests):

  Consider further testing in relation to potential for human exposure, e.g. where there may be widespread consumer exposure to significant amounts of a substance, a third test, preferably, *in vitro*, may be considered necessary for added assurance. Structure Activity Relationships (SAR) may be of use.

Note 1: The suggested approach is based on the assumption that any positive *in vitro* result should be tested *in vivo*. Also that when a chemical gives a positive result in somatic cells *in vivo*, there is no need for further testing for somatic cell effects *in vivo*, but testing for potential germ cell effects should be considered, and the chemical should be regarded as a potential human carcinogen.

Note 2: In order to "negate" positive *in vitro* data, *in vivo* data from at least two different tissues will be needed. A negative result in the first tissue (invariably the bone marrow) will not (under any circumstance that we can envisage) be adequate.

Annex 1

Guidance for the Use of Uncertainty Factors

Introduction

1. In the context of initial assessments, the Estimated Dose of Low Concern (EDLC) on repeated-dose toxicity and reproduction/developmental toxicity can be derived by dividing a N(L)OAEL by an Uncertainty Factor (UF) and compared to the Estimated Human Exposure (EHE).

2. Uncertainty Factors (UF's) mentioned in this Annex are used in the context of being equivalent to "safety factors", "assessment factors", etc., which are generally applied to results from animal experiments to increase the confidence that recommended exposure concentrations for humans, if realised, do not lead to adverse effects in the majority of people exposed. Thus, in general, the more relevant the available animal data are to the human situation, e.g. if the same exposure route is anticipated, the smaller the UF. These UF's are only guidance values for use in assessing consumer/general public exposure. They are not intended for occupational exposure for which lower values may be appropriate.

3. UF's will not be used in the OECD context; however, the approach can be used for any considerations at a national level.

Uncertainty Factors for Repeated Dose Toxicity

4. Traditionally, factors of, for example, 10 x 10 for inter- and intra-species variations have been used. Intraspecies variation refers to the differences in sensitivity within the same species (e.g. differences due to age), while interspecies variation refers to the differences in sensitivity between humans and animals. This has been a workable approach to deal with the uncertainties inherent in the extrapolation process, and is often based on the NOAEL from a study with an exposure duration of at least 90 days. However, the reliance on such pre-determined values may be misleading in terms of the confidence placed in the test results. UF's are guidance values only, and consideration should be given to increasing or decreasing their value on a case-by-case basis depending on the confidence that can be placed in the value of NOAEL. Factors affecting this confidence are the duration of the study, the dose-response relationships, the nature of the effects observed, the spacing between the doses, the quality of the toxicity data, and the type and severity of toxic effects observed. In one Member country, based on a 28-day test, in addition to an UF of 100, a factor of 10 is used to extrapolate to 90-day studies and an additional factor of 10 to extrapolate from such studies to long-term studies.

5. The following ranges UF's are suggested for guidance when applied to the:

- NOAEL: 100-300/500
- LOAEL: 500-1500/2500

These UF's cover the values generally used in Member countries, which might also include the value for the consideration of the worst case. It should be realised that UF's are always arbitrarily chosen and are not supported by strong scientific evidence.

Uncertainty Factors for Reproduction/Developmental Toxicity

6. UF's which are suggested to obtain an EDLC for reproduction/developmental toxicity may be different from those for repeated dose toxicity. The use of a relatively high UF is suggested when severe or irreversible developmental effects occur at exposure levels below those that induce well-defined parental toxicity, in other words when the test substance produces severe developmental effects that are not secondary to general toxicity. The same UF may be applied when developmental toxicity is only observed at exposure levels that are also toxic to the parent animals. The same considerations that went into the selection of the UF for the repeated dose study should be addressed when selecting the UF for allocating an EDLC for reproductive/developmental toxicity. An assessment of whether the effects seen in reproduction toxicity studies are secondary to general toxicity, or are specific reproductive effects, is important and expert judgement is necessary.

7. As in the case of repeated dose toxicity, an additional uncertain factor of 5 is suggested when a NOAEL cannot be obtained in the study and a LOAEL has to be used. Depending on the nature of the effects, the dose-response relationship and the spacing between the doses in the study, an even higher factor might be used.

8. UF ranges which are suggested as guidance for the NOAEL are:

a) parental toxicity, impaired fertility and reproduction toxicity	100-300/500
b) NOAEL for developmental effects lower than the NOAEL for maternal toxicity	1000-3000/5000
c) NOAEL for developmental effects higher than, or equal to, the NOAEL for maternal toxicity	100-300/500

9. Again, they cover the values generally used in Member countries, which might also include the value for the worst case analysis. As stated for the UF's for repeated dose toxicity, it should be realised, however, that UF's are always arbitrarily chosen and although this approach has been used for many years it is not supported by strong scientific evidence .

10. This concept of using more stringent UF's for developmental effects when they occur in the absence of maternal toxicity than when observed at levels that are also toxic to the parent animals is generally accepted. However, individual Member countries may consider developmental toxicity in the presence of maternal toxicity of as great a concern as developmental toxicity seen in its absence.

Annex 2

References

1. Relevant Monographs of the IPCS

- Environmental Health Criteria document (EHC) No. 6, Principles and Methods for Evaluating the Toxicity of Chemicals, 1978.

- Environmental Health Criteria document (EHC) No. 70, Principles for the Safety Assessment of Food Additives and Contaminants in Food, 1987.

- Environmental Health Criteria document (EHC) No. 104, Principles for the Toxicological Assessment of Pesticide Residues in Food, 1990.

- Environmental Health Criteria document (EHC) No. 170, Assessing Human Health Risks of Chemicals: Derivation of Guidance for Health-based Exposure Limit, 1994

2. Reference for "margin of safety" approach

- Technical Guidance Documents in Support of the Commission Directive 93/67/EEC on Risk Assessment for New Substances and the Commission Regulation (EC) N^{O} 1488/94 on Risk Assessment for Existing Substances, European Commission (in print)

3. Reference for Uncertainty Factors

- V.J. Feron, P.J. van Bladeren and R.J.J. Hermus, A Viewpoint on the Extrapolation of Toxicological Data from Animals to Man, Fd Chem Tox, Vol 28, No. 11, 783-789, 1990.